

WHAT IS CLAIMED IS:

- 1           1. A method of creating a library of DNA sequences, said method  
2 comprising:
  - 3           a) providing a DNA sequence that encodes a protein of interest;
  - 4           b) providing a probability matrix for the protein;
  - 5           c) providing a constraint vector for the protein;
  - 6           d) applying the constraint vector to the probability matrix to produce a  
7 substitution scheme recommending substitutions at at least two residues in the protein;  
8 and
    - 9           e) creating a library of DNA sequences incorporating changes in the  
10 DNA sequence that produce the recommended substitutions.
- 1           2. The method of claim 1, wherein said protein is selected from the  
2 group consisting of an esterase, dehydrogenase and hydrolase.
- 1           3. The method of claim 2, wherein said protein is selected from the  
2 group consisting of a protease, cellulase, lipase, hemicellulase, laccase, and amylase.
- 1           4. The method of claim 1, wherein said protein is selected from the  
2 group consisting of a transcription factor, growth factor, antibody, interleukin, antigen,  
3 and receptor.
- 1           5. The method of claim 1, wherein the probability matrix is based on  
2 structural characteristics selected from the group consisting of conservative residues,  
3 sequence alignments, three dimensional structure, residue environment, solvent  
4 accessibility, residue chemistry, propensity for a particular secondary structure, and  
5 combinations thereof.
- 1           6. The method of claim 1, wherein the constraint vector is based on  
2 structural characteristics known to affect protein function selected from the group  
3 consisting of proximity to the site of functionality, distance of  $\alpha$  or  $\beta$  carbons, contact  
4 with residues of interest, and contact with residues that contact the residue of interest.

1                   7.     The library of claim 1, wherein said library is a phage library.

1                   8.     A method for screening a library for a protein with an increase in a  
2     property of interest, comprising:

3                   a)     providing a probability matrix for a protein of interest;

4                   b)     providing a constraint vector for the protein;

5                   c)     applying the constraint vector to the probability matrix to produce a  
6     substitution scheme recommending substitutions at at least two residues in the protein;

7     and

8                   d)     creating a library of DNA sequences incorporating changes in the

9     DNA sequence that produce the recommended substitutions; and

10                  e)     screening the library for a protein with an increase in the property  
11     of interest.

1                   9.     The method of claim 8, further comprising identifying a protein

2     having an increase in the property of interest.

1                   10.    A protein produced by the method of claim 9.

1                   11.    A system for creating libraries of nucleic acid sequences that

2     encode variants of a protein, said system comprising:

3                   a)     an initial nucleic acid sequence that encodes a desired protein;

4                   b)     a probability matrix; and

5                   c)     a constraint vector.

1                   12.    A method for improving a desired parameter of a protein of

2     interest, comprising:

3                   a)     providing a probability matrix for the desired protein;

4                   b)     providing a constraint vector for the desired protein;

5                   c)     applying the constraint vector to the probability matrix to produce a  
6     substitution scheme recommending substitutions at at least two residues in the protein;

7     and

8                   d)     creating a library of DNA sequences incorporating changes in the

9 DNA sequence that produce the recommended substitutions; and  
10                   e) measuring the parameter of interest for at least two members of  
11                   said library;  
12                   f) determining the sequence for at least two members of said library;  
13                   and  
14                   g) using sequence comparison and correlation analysis to determine  
15                   the contribution of mutations or combination of mutations on the parameter measured in  
16                   step e).

1                   13. The method of claim 12, wherein the contribution of mutations  
2 determined in step g) is used to generate a second library.

1                   14. The method of claim 1, wherein a library comprising at least 25  
2 unique DNA sequences is produced.

1                   15. The method of claim 14, wherein a library comprising at least 100  
2 unique DNA sequences is produced.

1                   16. The method of claim 15, wherein a library comprising at least 250  
2 unique DNA sequences is produced.

1                   17. The method of claim 16, wherein a library comprising at least 1000  
2 unique DNA sequences is produced.

1                   18. The method of claim 17, wherein a library comprising at least 2500  
2 unique DNA sequences is produced.

1                   19. The method of claim 18, wherein a library comprising at least  
2 10,000 unique DNA sequences is produced.

1                   20. The method of claim 1, wherein a library of less than  $10^9$  unique  
2 DNA sequences is produced.

1                   21. The method of claim 20, wherein a library of less than  $10^6$  unique  
2 DNA sequences is produced.

1                   22. The method of claim 21, wherein a library of less than  $10^5$  unique  
2 DNA sequences is produced.

1                   23. The method of claim 1, wherein the probability matrix is an